

ST: Almost confluent growth of very small colonies; perhaps enough ST^r present so inoculum should have been smaller.

CG 9: SM: Light background growth + ca 800-1000 resistant papillae per plate. Papillae are of both the smooth & rough (or dense and translucent) colony types.

ST: Very light background; no papillae as yet.

6/1/54

Remover plates: Only change over night; response of 2 UV2 more clear cut

A3 +

A4 +

HC ++

All others -

Would have expected CG 7+8 to respond to A1 as they do in liquid media.

CG 9 S^r: All 4 col. picked as possible S^r growing well on AG2 SM.

Other antibiotic plates (now 3 days)

CG 9 ST: No change; no papillae - background very light

CG 7 SM: Growth so heavy, suggests this culture may already be S^r . Check slant

CG 7 ST: Papillae almost confluent; picked a few of largest papillae & streaked on St medium. However, if this represents normal rate of mutation to St^r , this marker would be very useful. Check slant - streak out & test individual colonies; if St^s , grow in broth & spread plates.

6/2/54

Run down plates; CG 7 & 8 now well grown on

H.C.; faint growth of these & of 2 UV 3, 4, & 5 on YIVA.

CG 9 S^r : Pick 1 col.; streak on AG-1 & SM; pick & test several of resulting col. for S character; save 1 pure S^r .

Need testing SM & St resistance of all auxotrophs by cross-bushing on AG-1 & SM or St.

CG 7	2 UV 1
8	2
9	3
	4
	5
	6

5/3/54

Final readings on recond plates (5 days)

	<u>0</u>	<u>A1</u>	<u>A2</u>	<u>A3</u>	<u>A4</u>	<u>A5</u>	<u>HC</u>	<u>VITS</u>	<u>YNA</u>	<u>Na₂S</u>
CG 7	-	-	-	-	-	-	+	-	-	-
8	-	-	-	-	-	-	+	-	-	-
9	+	+	+	+	+	±	⊖	⊖	+	+
2UV1	+	+	+	+	+	+	+	⊖	+	+
2	-	-	-	±	±	+	+	-	-	-
3	+	+	+	+	+	±	+	⊖	+	+
4	-	-	-	-	-	-	±	-	-	-
5	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-

6/4/54

Tests of autos for S & SE sensitivity by cross-breaking

	<u>S</u>	<u>SE</u>		<u>S</u>	<u>SE</u>
CG 7	R	R	2UV1	S	S
8	R	R	2	S	R
9	R	S	3	R	S & R
			4	R	R
			5	R	R
			6	R	R

However, spread plates indicate that CG 7 is not uniformly S & R nor CG 9, uniformly S & R. CG 7 did appear to be uniformly S & R. CG 7 has been checked out on A6-1; pick & test at least

10 col. for *St. nactini*.

6/5/54

Pinked col. of CG7, cross brushed \bar{c} *St.*

CG9; 2 UV1 and 3; Check for anaerotrophy.

Try spotting on minimal

2) Growing in minimal tubes,
 \bar{c} & \bar{s} aeration.

Checked motility of aerotrophs (microscopically from rather old plate). Under these circumstances only CG7 and 8 motile. All should be checked in soft agar.

6/7/54

CG7 single colonies/*St.*; all 10 *St.*^R.

CG9, 2 UV1 & 3; Failed to grow in aerated or non-aerated min. tubes (2 days). On min. plate, growth very scanty. Unfortunately failed to spot w.t. as control on plate.

6/8/54

Made random plates - single omissions of AA groups - to check 2 UV3, 4, & 5. Will also include other

cupos, since none of the requirements is completely determined

Spotted cupos on AG 1 for repl. to sundrows

6/9/54

Cupos on AG-1 (24 hrs); Good growth of CG 7, 8 + 9; 2UV1, 2, + 3; fair growth of 4, 5, + 6. Replicated to AA group single omission sundrows

6/10/54

Strucked all CG and radiobacter cultures on AG-1 \bar{c} aniline blue added (1:10,000).

6/11/54

Examined galls (4 weeks). See results on next page. Saved plants for recovery of organisms from galls.

In plants in 1-14+5 series, where one +, for example is indicated, there were usually one or 2 good galls and very little hypertrophy at the other inoculation sites. In other cases (purified cultures - A6 derivatives) galls were quite uniform at all sites.

Doubtful auxotrophs spotted on AG-2 June 5'
 Moderate growth (CG 9, 2UVI & 3) Should
 have used prototroph control. Still no
 growth in minimal tubes inoculated at the
 same time.

Experiments replicated 6/9

	0	-A1	-A2	-A3	-A4	-A5	AA+	HC	Y.EX	complete
CG 7	-	±	+	+	+	+	+	+	+	+++
8	-	±	+	+	+	+	+	+	+	+++
9	±	±	+	+	+	+	+	-	++	+++
2UVI	±	-	+	+	+	+	±	+	+	+++
2	-	+	+	+	+	+	±	+	+	+++
3	±	±	+	+	+	±	+	+	+	+++
4	-	-	±	±	±	±	-	-	+	++
5	-	-	±	±	±	±	-	-	+	++
6	-	-	±	±	±	±	-	-	+	++

Note: this is the second time. CG 9 has shown
 anywhere from a trace to good growth on minimal
 and a variety of supplements, but no growth on
 minimal + HC.

Attempt isolations from circled plants.

Macerate galls in sterile H₂O, streak heavily on
 B glu and AG-1 + on pl.

Plant readings
6/11/54

control

000

CG 5 (+)

" +

" ±

CG 4 +++

+++

+++

CG 3 (+++)

+++

++

CG 1-R + (poor growth of plant)

++

(++)

CG 2 M ±

" ±

" ±

CG 1 M (+++)

+++

+++

AG UV-1 0

(+)
0

1M+5 R1 ± (one good fall)

1M+5 R1 +

" R4 +

1M+5 (R2) +

When through plants,
cut tops off & throw
in garbage can; dirt
in large cans near
door.

" M3 +

R2 ±

M3 +

R2 +

(M3) ++

M4 ±

M2 +

M4 ±

M1 +

M4 +

M1 ++

M2 ±

M2 +

M1 ±

6/12/54

At 2 days, no absorption of aniline blue in any of cultures used.

6/14/54

Made attempts to recover bacteria from galls, as listed on previous page. Macerated slice of gall tissue in 2-ml saline, streaked on BB-1 at

Readings - final reading (repeated 6/9)

	<u>0</u>	<u>A1</u>	<u>A2</u>	<u>A3</u>	<u>A4</u>	<u>A5</u>	<u>AA</u>	<u>HC</u>	<u>YEX</u>	<u>Complete</u>
CG 7	-	±	++	+++	+++	++	++	++	+	+++
8	-	±	++	+++	+++	++	++	++	+	+++
9	±	+	+	+	+	+	+	-	+	+++
2UV1	±	±	++	++	+	+	+	+	+	+++
2	-	++	++	+++	+	+	++	+	+	+++
3	±	++	++	++	+	+	+	+	+	+++
4	-	-	+	+	+	±	+	+	+	++
5	-	-	+	+	+	+	±	+	+	++
6	-	-	+	+	+	+	+	-	+	++

Being fed at one edge by mixed colony.
Note that most cultures grew best in the absence of A3, and CG 7 & 8, in the absence of A4.

2UV 4, 5, & 6 Try on A1 alone & various other samples, singly. Also try this on CG 7 & 8. In this expt,

CG 9 & 20V143 grew little if any and unsufficiency
 minimal, in contrast to previous experiment.

6/16/54

Isolations from galls: After 2 days, all plates
 have colonies predominating that look like
Agrobacterium. Some col. from galls inc. to
 SR (CG 4 + CG 5) absorb aniline blue
 strongly.

Pick at least 10 col. each. Test for prototrophy/
 auxotrophy and SA. Save for tests of
 virulence. CG-1-R as reserved is still
 rough.

Save actinomycete on 1-M+5 (M3) plate for J. L.

6/17/54

At 3 days, the col. from CG-4 that absorbed aniline
 blue are very large, gummy, and yellow. Test
 one of these and one ordinary, colorless colony for
 SA resistance and auxotrophy. The blue-absorbing
 col. from CG-4 did not become yellowish or
 very gummy.

Now, on 3rd day, more contaminants are showing up in streaks; most are highly pigmented, yellow, orange, and pink.

R2 from mixture experiment came out smooth, unlike CG-1-R, which was rough when recovered. This probably indicates that the organisms causing the gall were from the CG-1-M used in the mixture, carried over as a contaminant when SR growth was pecked, or perhaps a mutation to SR in CG-1-M.

For consistency, CG-1-M and CG-2-M should be called smooth, rather than mirrorid, because they are not unusually gummy for this organism. As of now, CG-1-M = CG-1-S;
CG-2-M = CG-2-S.

Made up a batch of AG-1 agar using 0.5% mannitol instead of 0.5% sucrose to see whether a different carbohydrate would make any difference in indole blue absorption.

Made up 20x salts solution for AG-2, incorporating Fe & Mn in soln. (from conc. stock Fe & Mn salts).

6/18/54

Some streaks from gall isolations not yet grown up enough to pick. Continue inc. for another day.

6/19/54

Spotted all auxos on complete for replication to AA's + AA1. Stored supplemented plates in frig.

Picked 1 col from each streak from 6/15/ plates of gall isolates.

CG-1-S: some streaks have a few R-type col.

CG-1-R: all R type col, except 1 streak mixed

S & R

CG-4: 3 of the ten streaks are yellowish, with intense blue color in the medium. The remainder are ordinary white S colonies.

CG-5: One streak - no growth; of remaining 9, one has blue colonies & intense blue coloring in medium. Blue colonies are small and not gummy.

CG-9: White, S-type col. Some streaks have a very few R.

X M3: 1 yellow, very gummy; 2 moderately gummy remainder less gummy and rather translucent.

X R2: All colonies moderately smooth; not so smooth

CG-1-S

Spotted each suspension on complete for replication
to minimal + SM.

6/21/54

used half the
conc. added for E. coli
(0.1 mg/ml)

Replicated gal re-isolates to AG1, AG (SM) & AG2
(3 of the isolates from CG4 & 2 from X173 are
very yellow & gummy; check identity)

Replicated auxotrophs to complete, mini, and
AI + other AA groups singly.

6/22/54

Put in slabs:

CG-1-S

CG-1-R

CG-2-S

CG-2-R

} Have slabs only of A6 and A6-6 as
received (mixed S & R)

CG-7

2UV-1

2UV-4

CG-8

2

5

CG 9

3

6

In plant experiment, inocula taken from mixtures
were put on slants as well as inoculated onto
plants. Struck out R2 & M3, pick 10 or more
colonies, test for purity & respect to SM.

6/23/54

All isolates

	<u>Type of growth</u>	<u>SM</u>		<u>Type of growth</u>	<u>SM</u>
CG-1-S	10 S	10 S ^S (see note)	CG 9	10 S	10 S ^R
CG-1-R	2 S, 8 R	10 S ^S	X M3	10 S	2 S ^S , 8 S ^R
CG-4	3 S yellow, 7 S white	10 S ^R	X R2	10 S	10 S ^R
CG-5	8 S, 1 R	9 S ^R			

S^S more common than S^R though all are smooth type of growth.

CG-1-S : all S^S, but all spots have a few S^R colonies (2 damp) . CG-1-R : no growth at all on SM.

All isolates grew very well on minimal.

The three yellow isolates from CG 4 are bright yellow and extremely gummy; check microscopically.

Randoms inc. 6/21

	<u>0</u>	<u>A1</u>	<u>A1+2</u>	<u>A1+3</u>	<u>A1+4</u>	<u>A1+5</u>	<u>A1+HC</u>
CG 7	-	-	-	-	-	-	+
8	-	-	-	-	-	-	+
9	-	-	-	-	-	±	±
2 UV-1	±	±	±	±	±	+	+
2	±	±	±	±	+	±	+
3	±	±	±	±	±	±	+
4	-	-	-	-	-	-	±
5	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-

Might be able to try some SRP (or St^RP) experiments, using the following cultures. All are apparently stable auxotrophs, although their requirements are elusive.

	<u>S</u>	<u>SE</u>	<u>Source</u>	<u>Arrangement of test plates</u>	
CG 7	R	R	CG-2		
CG 8	R	R	CG-2	1 S	2UV1
				1 R	3
2UV-2	S	R	CG-1	2 S	4
2UV-4	R	R	"	2 R	5
2UV-5	R	R	"	7	6
				8	
2UV-6	R	R	"		

Check these and CG-1 S & R and CG-2 S & R by cross brushing \bar{c} SM & St. If the prototroph used is S^SSt^S, one of these markers could be used, ^{possibly} along \bar{c} virulence, as an unselected marker.

In testing SM & St status, include 2UV1, previously recorded as S^SSt^S, as control.

Steps - CG 7 stab appears to be contaminated (yellow).
Inoc. new stab from slant.

6/24/54

Pick colonies from streaks of XB2 and XM3 from slants; restreak.

Randoms mic. 6/21:

	<u>0</u>	<u>A1</u>	<u>A1+2</u>	<u>A1+3</u>	<u>A1+4</u>	<u>A1+5</u>	<u>A1+HC</u>
CG 7	±	±	±	±	±	±	++
8	±	±	±	±	±	±	++
9	-	-	-	-	-	-	⊕
2UV1	±	±	±	±	±	±	+
2	±	±	±	±	+	±	++
3	±	±	±	±	±	±	+
4	-	-	-	-	-	-	±
5	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-

CG 9 has always failed to show even a faint trace of growth on HC alone.

Need a better minimal medium.

Try medium based on Hodgson, Peterman, & Piller, 1951

$(\text{NH}_4)_2\text{SO}_4$ 1.0

NaCl 0.2

$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.1

$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2

$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 8.8 mg

ferric alum 43 mg

$\text{Mn}_2\text{SO}_4 \cdot 4\text{H}_2\text{O}$ 0.8 mg

Use $\text{K}_2\text{H}_2\text{PO}_4$ 0.2 g/l
 KH_2PO_4 0.1

per liter

+ sucrose 20 g/l for liquid
5 g/l for agar

+ 40 ml pH 7.0 phosphate buffer per liter

Use Mn & Fe solns as made up - Use 1:200

Make up ZnSO_4 soln to be used 1:1000

0.176 g / 20 ml stock soln.

This differs from present medium chiefly in N source ($(\text{NH}_4)_2\text{SO}_4$ instead of KNO_3); also has less CaCl_2 and includes zinc.

Transferred to slants an example of each kind of isolate from galls - 1 per gall where all appeared identical; an example of each type when there were 2 or more types from one gall.

6/25/54

6/21 rundowns:

	<u>0</u>	<u>A1</u>	<u>1+2</u>	<u>1+3</u>	<u>1+4</u>	<u>1+5</u>	<u>1+HC</u>
CG 7	±	±	±	+	+	±	++
8	±	±	±	+	+	±	++
9	±	-	-	-	±	-	±
2UV 1	±	±	±	±	±	±	+
2	±	±	mold	±	+	±	+
3	±	±	±	±	±	±	+
4	-	-	-	-	±	±	+
5	-	-	-	-	±	±	±
6	-	-	-	-	±	±	-

Checks of streptomycin and streptothricin resistance:

	<u>S</u>	<u>St</u>		<u>S</u>	<u>St</u>
CG-1-S	S	R	2UV1	S	S
1-R	S	R	3(?)	S	R
2-S	S	R	4	S	R
2-R	S	R	5	R	R
7	R	R	6	R	R
8	R	R			

Hence St not useful to distinguish between proto. & aupos. [2UV-4 had been scored S^R on previous test]

2UV6 looks like a good aupos to try in SRP test.

Problem: How make sure it is really A. tumefaciens?

- 1) Microscopic
 - 2) Growth in complete broth, aerated and anaerobically.
 - 3) Growth at room temp + at 37°
- (For (2) & (3), use CG-1-S & R for comparison).
- 4) - Eventually - plant inoculation.

6/26/54

2UV6, CG-1-S, & CG-1-R: Aerated; Pump was turned off sometime during evening, so don't know how long cultures were actually aerated. CG-1-S aerated in

very much heavier than un-aerated; slight difference between 1-R aerated and un-aerated; and little or no difference between 2UV6 aerated and un-aerated. (ca 20 hrs)

Growth at 37°: CG1 S & R - slight growth; 2UV6 very little growth. Growth of all un-aerated cultures at room temp much better than at 37°.

Yesterday made up 5 plates AG1 @ 1:10,000 aniline blue (Coleman-Bell). Have been using National Aniline, same conc. Natl Aniline → pale greenish blue medium; Coleman-Bell → grayish purple.

Streaked CG1 S & R on plate made @ Coleman Bell dye.

Motility check on 2UV6: Motility about the same as CG1-R; higher proportion of motile cells than CG1-S. (Observations on 20-22 hr cultures, AG-1 broth, room temp.)

Inoc. both cultures of 2UV6 and CG1-2-S, together and separately, to be inc. at room temp @ +5 aerations.

X M3 & X R2 from slants (1-5) + 5 replicates each.
 Picked 1 col from each strake (12 from each culture),
 spotted on AG-1 for replication to SM.

6/28/54

Test of Gram - Cell outline Blue; Neither CG-1 or
 2UV-6 colored on AG-1 with dye 1:10,000.

2UV-6 identity. Have reservations about this culture
 being Corynebacterium because:

- 1) Cells appear slightly smaller than CG-1
- 2) CG-1 grows slightly at 37°; 2UV-6 not
 at all
- 3) CG-1 grows poorly in AG-1 broth \bar{c} aeration
 2UV-6 grows almost as well \bar{c} as \bar{c} aeration
 (Final opacity of culture about the same, but
 requires 48, rather than 24 hrs. CG-1
 never does grow very well in un-aerated
 broth).

X M3 & X R2 ^{from slants} Replicate to AG-1-SM.

Plated CG-2-S / 2UV-6 crosses on AG-2-SM.

Grown together - aerated Grown separately - aerated

Grown together - not aerated Grown separately - not aerated

2 UV-6 - In further check on identity,
streak on EMB sugar media, see if
it ferments any sugars.

6/29/54

X R2 & X M3 from slants; All 12
colonies tested from streak of each are
S^R - no evidence of mixture. (Though
of course S^R could come from mutation in
CG-1-S, rather than from CG-5.

S^R P plates (now only ca 20 hrs)

Controls: 2 UV6 aerated & un-aerated, no growth.

CG-2-S un-aerated; moderate background
film, a few papillae (S^R mutants?)

CG-2-S aerated; moderately heavy background
growth, no S^R papillae.

"Cross" plates

- 1) Grown together, aerated; Moderately heavy
background film, possibly some papillae.
- 2) Grown together, not aerated; Less heavy
background film, many papillae.
- 3) Grown separately, aerated; Moderately heavy
background, no papillae.
- 4) Grown separately, not aerated; moderate
background, many large papillae.

When papillae better grown, quite
test for autotrophy. Probably autotrophs being fed by background
of CG-1-S. If prototrophic - !!!

A36

Too early to try reaching any conclusions. Results
observed so far could be explained by feeding of
20V6 by background growth of CG-2-S; in
aerated cultures, the background growth of CG-2-
may be heavy enough to obscure papillae.
This growth of CG-2-S seems to be due to
too low S^M conc, rather than confluent growth
of S^R mutants, since papillae are readily
seen on plates spread in un-aerated cultures.
S^M conc. critical; at twice the conc. used,
growth of S^R cultures is inhibited.

Next - try "cross" using S^R auxo from CG-2,
so a "normal" CG-1 can be used, and
virulence of any resulting colonies can be tested.
(Quite likely that S^R auxo from CG-1 may have
lost its virulence - this apparently happened in
CG-9).

Use CG 7 (from CG-2) and CG-1-S.

Grow as before, mixed & separately, aerated &
un-aerated. Also mix extra mixture tube,
inc. in aeration for several days, rather than
only 48 hrs.

Again - identity of auxo. as Agrobacterium is not
definite.

6/29 1:30 pm 6/29

Picked 4 col. each 2-S/2UV6 "crosses" grown together & grown separately & plated together. Streaked on AG 2 SM.

6/30/54

SRP plates:

Controls:

2UV6 aerated and un-aerated: no growth

CG-2-S aerated; Moderate background growth; one large colony; many small papillae (thousands).

CG-2-S un-aerated; Heavy background growth (confluent growth corresponding to small papillae on plate from aerated culture?) ca 100 large papillae, of which 10 are as large as normal colonies.

"Cross" plates

1) Grown together, aerated; Rather heavy confluent growth. About 100 large papillae per plate (4 plates); About 10 per plate are the size of normal colonies. On 2 of the plates the background is not quite so heavy as on the other two, and there are thousands of small papillae.

One plate of the 4 has what appear to be

phage plaques in the area of heaviest growth. The plaques are present only on the portion of the plate near two large mold contaminants. (Molds supplying factor needed for phage growth??)

- 2) Grown together, not aerated: Moderately heavy confluent background growth. Many more large & moderate-size papillae than on plates from aerated culture. ("Colony-size" papilla on 4 plates 25, 30, 60, & 70).
- 3) Grown separately, aerated: Light to moderate background; thousands of tiny papillae; on 4 plates, total of only 3 large papillae.
- 4) Grown separately, not aerated: Moderate background; a few tiny papillae; rather large number of medium and large papillae ("Colony-size" papillae on 4 plates: 44; 29; 12; 58).

Colonies picked from "cross" plates & streaked on AG-2SM
 no growth in 24 hrs. Continue incubation. Also:
 check SM level of new batch of AG-2SM by streak
 on CG-2S and CG-5 (or CG-2-S & CG-3 or 4)

Pick more papillae from "cross" plates, streak on AG-1
 (no SM). Pick colonies from streaks, spot on
 complete, repl. to minimal & SM.

Phage: Scrape up growth and plaques, add to
AG1 broth. Also add to cultures of CG-2-S and
2UV6 in broth. 10:10 AM. Started aerating cultures
of 2UV6 and CG-2-S in AG1; also growth & plaques scraped
from plate. 1:15. Added 0.1 ml broth from culture from plate to
each of other two tubes.

Cultures for CG-1-S / CG7 experiment; CG-1-S
growing rather poorly, both in aerated and un-aerated
culture. However, should be OK to use at 48
hrs.

7/1/54

2S / 2UV6 SRP expt. i: papillae which were
tiny yesterday now full-size colonies, evidently
SR mutants of CG 2S, since nos. are about the
same on "cross" plates & CG 2S control plates.

About 200-300 per plate from un-aerated inocula;
several thousand from aerated inocula, which gave
heavier suspensions for plating.

Discarded plates. (Most were moldy)

Phage

✓ 1) Aerated cultures: a) mix direct from plate; b) 2UV6
+ mix from plate; c) CG 2S + mix from plate.

None show evidence of lysis. Cross-brush
each on complete med. c 2UV6 & CG 2S.

2) EMB plates streaked c 2UV6 (6/28); 2 kinds

of colonies, one very translucent, one more dense.
 Could this difference be due to phage? Pick, streak
 out (on EMB), save 1 col. of each type.

✓3) Save mold from plate on which plaques were
 found, in case cannot get plaques on ordinary
 complete medium. \bar{E} mold present.

4) Spread plates of complete medium \bar{E} mixture of
 CG2S and 2UV6.

CG-1-S/CG7 SRP \bar{E} pl: Cfg, wash, plate. SRP's picks
 from previous SRP experiment are growing rather poorly
 in ^{present batch of} AG2SM; probably had the usual E. coli con
 of SM added.

7/2/54

Plaque picked from 2S/2UV6 SRP plates: Streaked on
 complete \bar{E} 2 kinds of colonies: Small, translucent; a
 larger, opaque. Check both for asymmetry & S resistance
Pick to complete repl. to min x SM.

"Phage" This may not be a phage at all. The plates
 spread on \bar{E} CG2S + 2UV6 have what look like
 temp. \bar{E} colonies in large numbers, but the plate

streaked the previous day with material scraped directly from "plaque" area on original plate has two kinds of colonies:

- 1) Rather large, opaque (these were visible yesterday).
- 2) Tiny, transparent. - These little, water-clear colonies are not overgrown by the larger colonies, but when surrounded by a developing opaque colony, show up as round clear areas which one would interpret as plaques were it not for the other small transparent colonies on the plate.

Make further attempts to run down requirements of auxotrophs. Find out smallest conc. of YE which will permit ^{good} growth when added to minimal tubes; then check for inhibition by AA groups, urea, HC, etc.

Try some "crosses" of various auxotrophs to see whether they will grow on minimal either by recombination to form prototrophs or by syntrophism.

and streak 5% yeast extract to 10 ml tubes of AG 2, 0.1, 0.2, 0.4 & 1.0 ml per tube. Inoculate CG 9 and 20V1. CG 9 because it has shown a reaction

specific reference to YE and YNA, and latter because it is S⁺, the only one among the aerotrophs, and thus has an additional marker for crosses.

Also incubated AG1 broths of CG9 and 2UV1 together & separately for crossing attempt. To be plated on minimal. Did not start aerated cultures this time.

7/3/54

CG-1-5 / CG-7 SRP: (2 days)

Cultures:

7, not aerated: Thin film, no papillae

7, aerated:

1-5, not aerated: fairly heavy background,
16 ^{large} papillae

1-5, aerated: Heavy background, no papillae

Mixed together, not aerated: fairly heavy background, ca 50-100 papillae per plate. Very clear areas, either plaques or transparent colonies.

Mixed together, aerated: Heavy background, few papillae, no "plaques"

Mixed separately, not aerated: Moderate background, 50-100 large papillae per plate

Mixed separately, aerated: Moderately heavy background,

only 2-4 papillae per plate.

On checking back to plate on which "phage" appeared - the "plaques" on that plate do not look like the effect produced by the small transparent colonies. They ^{the "plaques"} have rather ragged edges and turbid centers. Save plate, try again to get a phage from it.

7/5/54

Plate spotted \bar{c} two col. types from 25/20V6
(1/2) Moldy; spot on fresh A61 plate for replication
to men & S4.

~~Original "phage" plate; "plaques" still visible;
Try picking from individual clear areas, see whether
→ small, clear colonies; also inc in broth
20V6 + CG25 see if any phage~~

Look very similar to CG-15 plate - see below

CG9 + 20V1 in men. + yeast extract:

CG9: Good growth in 10 ml men + 0.1 ml 5% YEX.

(In remainder, this depo was recorded as responding to YVA).

20V1: Some growth \bar{c} 0.1, 0.2, & 0.4 ml YEX;
quite good growth \bar{c} 1.0 ml, but still not as

good as in complete. (\bar{c} CG 9, growth ≈ 0.1 in YEX is about as good as in complete). Growth in men + 0.4 ml YEX probably OK for testing for inhibition.

Add regular conc of into & AA groups to men + necessary amt of YEX.

CG-1-5 / CG 7 "SRP" ^{apf}; (plated 7/11)

Controls: 7 aerated & un-aerated; several hundred colonies; evidently this single conc not too satisfactory for "crossing" effects.

1-5, not aerated; moderate background which has "plagued" appearance - apparently due, again, to small, transparent colonies. Also ca 100 large papillae.

1-5, aerated; moderate background, several hundred papillae.

"Cross" plates: very similar to CG 1-5 controls.

20VI / CG 9: C₆ wash plate, ^{on men} (grown together for 30 min. ^{on men SM}). Also plate, ^{on men SM} mixture of CG 1-5 and CG 7 incubated 5 days. Not too promising.

an overview of behavior of controls.

7-5/7 Result about the same as those incubated only 2 days,
Heavy growth + numerous leakage particles

7/6/54

Cultures from 2S/2UV6 expt. Replicated to
AG2 + AG1517.

CG9 + 2UV1 - in min + YE + reagents (24 hrs)

	CG9 48h		2UV1			CG9 48h		2UV1	
0	-	-	±	-	YE+AA4	-	±	±	-
YE only	+	++	+	++	AA5	+	++	+	++
YE+AA1	+	++	+	++	HC	-	-	±	-
AA2	+	++	±	++	VITS	-	+	-	-
AA3	+	++	+	+	Na ₂ S	+	++	++	+++

better growth in
YE + Na₂S than in YE
alone.

Incubate at least one more day, then try individual
members of any AA groups which are inhibitory.

Try vitamin mix for inhibition of prototrophic
Agrobacterium and other growth factors.

Start this type of experiment with other auxotrophs

7/7/54

Test of "papillae" from 2S/2UV6 SRP experiment.

Papillae on minimal SMY picked

↓ streaked on complete

small,
translucent

larger,
opaque

6 col. of each
tested

all S^R,
auxotrophic

all S^S, prototrophic
(but inhibition on SMY
not complete -
hence gave background
which permitted auxotrophic
S^R (2UV6) to grow).

2UV1/CG9 "cross"; No colonies on controls
on cross plates. (2 days). Had to discard most
of cross plates because of mold.

Check on inhibition of prototrophs by growth factor solution
24 hrs: growth poor; detectable only in complete
AA3, and sucrose.

Perhaps would be a good idea to add Na₂S
routinely to minimal medium.

Also: try using (NH₄)₂SO₄ instead of KNO₃
as N source. See medium list p A33

7/8/54

Make up 20x salts for minimal, using only
 0.2 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ per 100 ml 20x soln
 (see p A33)

Then make up 2 media

1) 0.5% sucrose, 0.5% KNO_3 ; Fe & Mn
 (usual A₂, except for less CaCl_2)

2) 0.5% sucrose, 0.1% $(\text{NH}_4)_2\text{SO}_4$; Fe, Mn, Zn
 (~ Hodgson, Peterson, & Riker 1951)

ADD Na_2S to portions of each

CG9 and 2UV1 in min. + YE + renderous (3 days)

	<u>CG9</u>	<u>2UV1</u>		<u>CG9</u>	<u>2UV1</u>
0	-	-	YE+A4	++	-
YE only	+++	+++	A5	+++	+++
YE+A1	+++	+++	HC	-	-
A2	+++	+++	VITS	+++	-
A3	+++	+	Na_2S	+++	+++

CG15 and CG25 in min. + renderous (2 days)

	<u>CG15</u>		<u>CG25</u>			<u>CG15</u>		<u>CG25</u>	
0	+	+++	+	+++	A5	-	++	-	++
A1	+	+++	+	+++	HC	-	-	-	-
A2	+	+++	+	+++	VITS	-	-	-	-
A3	++	+++	++	+++	Na_2S	+++	+++	+++	+++
A4	-	-	-	-	complete	++	+++	++	+++

Inoculated CGIS into tubes as follows:
 1 Complete 3 A3 and components
 2 Minimal 4 A4 and components
 5 A5 and components

7/9/54

Discarded remaining plates of 2UVI/CG9 "crossing" experiment. 1 colony on 2UVI control; 1 colony on a cross plate. Both near molds. No other colonies

CGIS in min + AA groups + components:

	1 day	2 days	3 days		1 day	2 days	3 days
0	-	++	++++	glutamic	-	-	-
complete	++	+++	++++	aspart	-	-	contaminated mold, growth
A3	+	+++	++++	prol	+	++	++++*
trypt	±	++	++++	A5	-	-	++
tyros.	+	+++	++++*	alan	+	+++	++++
φal	+	+++	++++*	glyc	+	+++	++++
A4	-	-	-	serine	-	±	+++
hist	-	++	++++	OH prol	+	+++	++++
thres	±	+++	++++				

In tubes marked * there was very heavy growth in the upper 1/4 to 1/3 of the tube. Other tubes in which growth occurred were more uniform.

Prepared tubes of AG 2 (low $CaCl_2$) \bar{c} & 5 sulfide; also the $(NH_4)_2 SO_4$ - Zn^{++} medium (AG. 3) \bar{c} & 5 sulfide
 Inoc. \bar{c} CGIS

Attempted crosses of auxotrophs

	CG 7	8	9	2UVI	2	3	4	5	6
CG 7		7/5/54 1 on 8 control 1 on 9 control	7/5/54 0	7/26/54 ✓ 61; control 0					
8			7/5/54 0						
9				7/9/54 0					
2UVI									
2									
3									
4									
5									
6									

Start cultures of CG 7, 8, 9, 7+8, 7+9, & 8+9 for crossing exp

Inoc. 2 CG 15 tubes of minimal + each vitamin available in solution.

7/10/54

Test CG 15^R = individual acids of ^{AA} groups 1 and 2.
 (Used CG 1R by mistake - shouldn't make any difference in eventual result.)

7/10/54

CG 1S and CG 2S in mini + growth factors.

	<u>CG 1S</u>	<u>CG 2S</u>		<u>CG 1S</u>	<u>CG 2S</u>
0	+++	+++	A5	+++	+++
A1	+++	+++	HC	-	-
A2	+++	+++	VITS	-	-
A3	+++	+++	Na ₂ S	+++	+++
A4	-	-	complete	+++	+++

Discard

See on page 43 results of adding individual AA's.
 McIntire, Peterson, and Riker (1941) used glutamic acid at 2.5 gm per liter in their minimal medium!!! This was for the same strain (A6, Van Lanen, Baldwin, & Riker (1940) reported mono-amino-dicarboxylic acids inactive in attenuation (and presumably inhibition) up to 17/20

CG 1R in groups AA1 & AA2 and components of these groups. No growth at 20 hrs in mini. or any supplement.

CG 1S in Ag 2 & Ag 3 ± 45 sulfide - no growth at 24 hrs.

CG 1S in mini + individual ams - no growth 24 hrs.

7/11/54

CGIR in A1 + 2 + components:

	<u>2 days</u>	<u>3 days</u>		<u>2 days</u>	<u>3 days</u>
0	+	+++	A2	++*	+++
A1	++*	+++	lec	++*	+++
lys	++*	+++	isoleuc	++*	+++
arg	++*	+++	val	++*	+++
meth	++*	+++			
cyst	+	+++	3rd day; all have heavier growth near top of tube.		

* Growth heavy in upper portion of tube (not a pellicle)

CG-1-S in AG 2 + AG 3 \bar{c} + 5 sulfide

	<u>2 days</u>	<u>3 days</u>
AG 2	-?	- (?)
2+ Na ₂ S	++ (uniform turbidity)	+++
AG 3	-	-
3+ Na ₂ S	+ (bottom 1/2 of tube turbid)	+ (now uniform throughout)

~~REPEAT~~

CG 1 S in individual vials.

	<u>2 days</u>	<u>3 days</u>		<u>2 days</u>	<u>3 days</u>
0	++	+++	nei	+++*	+++
B6	++	+++	B1	+++	+++
Choline	++	+++	B2	++	+++
Mandelate	++	+++	pala	+++*	+++
Pentothamate	++	+++	essetil	+++	+++
	+++	+++	A12	+++	+++

7/12/54

CG 7, 8, & 9, pairs grown together & separately

Repeat of CG 15 in AG 2 + AG 3 \pm + S sulfide conc.
(May have been an error in conc. of AG 2 + 3 salts soln. If no growth in AG 2 this time make up new 20x stock)

7/13/54

Repeat on AG 2 + AG 3 \pm sulfide:

	1 day	2 days	3 days
2	-	-	-
2 + sulfide	-	-	-
3	-	-	-
3 + sulfide	+	+++	+++

Inc. AG 2 & AG 2 + folic acid (pteroylglutamic) \pm CG 15.

7, 8, & 9 crosses - Nothing at 24 hrs.

Try CG 15 in mini + glutamic or aspartic + sulfide

7/14/54

CG 15 in AG 2 ± PGA: nothing at 20 hrs.

Crosses involving 7, 8, & 9: nothing at 2 days except good growth around molds. A few plates appear uniformly seeded (in the agar) with old friend yellow contaminant.

7/15/54

CG 15 in AG 2 ± folic acid (PGA): equally good growth ± 0.5 PGA.

Crosses involving 7, 8, & 9: (3 days) - nothing definite. 1 possible prototroph on (7+8) plate. Plate contaminated ± yellow bug, but this colony buff & looks like typical CG. Also one buff colony on CG 8 control plate, but aside from color it looks just like yellow contaminant.

- Pick:
- 1) col from cross plate
 - 2) buff col from control plate
 - 3) yellow colony.

Streak out; test whether inhibited by glutamic acid.

7/16/54

could try
stuck water mix
dated 12/15/52
also try
+8 (w/)

Start repeat run-downs of all auxotrophs, avoiding any inhibitory supplements. (Do vials individually).

Made up new 20x AG 2 salts; previous batch gave no growth & sulfide

CGIS in AG 2 ± glutamic ± Na₂S (2 days)

AG 2	-	AG 2 + Na ₂ S	++
AG 2 + glutamic	-	AG 2 + g.a. + Na ₂ S	++

Try CG 2 S in run. This was earlier batch than that which gave no growth in previous exp.

Try run-downs of auxos ± Na₂S added as detoxifier

For some reason - new batch of AG 2 salts → no ppt in 2x concentration

NOTE: There is a large bottle of d-glutamic acid in the AA box, and much of it has been used. Could my present stock saline be d-glutamic? The aspartic acid on hand is dl, so possibly d some could explain inhibition by both glutamic and aspartic salts.

7/17/54

CG15 and 2UV6 in mediums + sulfide

no	CG 25			2UV6		
	1 day	2 days	3 days	1 day	2 days	3 days
Na ₂ S	-	+	++	-	-	-
Na ₂ S only	-	+	+++	-	-	-
A1	±	+	+++	-	-	-
A2	±	+	+++	-	-	-
A3	+	+	+++*	-	-	-
A4	+	+	+++*	-	-	-
A5	-	+	++	-	-	-
HC	++	++	+++	+	+	++
VITS	-	±	++	-	-	-
YEX	++	++	+++	+	++	+++

7/19/54

Prototypes from 7+8 cross & 8 control plate. Both grew very poorly on minimal (plate rather than tube). Colonies look identical; the one from 8 control plate does not resemble the yellow contaminant. Inoc each into min. tube & min + AA4 or glutamic acid.

Prepared the following tubes & inoc CG15

- min
 - min + l-glutamic
 - min + d-glutamic
- { min + d-glut. + Na₂S
 { min + " + thiosulfate
 (1:10,000)
 (as checks on whether thiosulfate is as effective as Na₂S)

Started run-ins + sulfide = CG 7 & CG 8

7/20/54

CG 15 in l-glutamic + in d-glutamic alone +
 = Na₂S + thiosulfate; no growth at 24
 hrs.

"Prototrophs" from 7x8 & 8 control; No growth at
 24 hrs in run-in or run-in + glut.

Randoms on CG 7 & 8 (black sulfide ppt in
 some tubes)

	CG 7				CG 8		
	1 day	2 days	3 days	4	1 day	2 days	3 days
Na ₂ S only	-	-	±	±	-	-	-
A1	-	++	++	+++	-	++	+++ +
A2	-	-	±	±	-	++	+++ +
A3	-	-	±	+	-	-	- -
A4	-	-	+	+	-	-	++ +
A5	-	-	±	±	-	-	+ +
HC	+*	+++	+++	+++	+*	+++	+++ ++
VITS	++?	?(-)	?(-)	-	++?	?(-)	?(-)
YEX	+*	+++	+++	+++	+*	+++	+++ +

* Growth out
 7/21: Turbidity in vitamin tubes apparently was
 ppt in medium; has largely disappeared.

CG 1 and 2 on 7/21

7/21/54

CG 15 in d- & l- glut. acid (2 days)

Mold growing in 0, d- glut only, & l- glut only tubes

0 ++

l- glut - d- glut + Na_2S +++

d- glut - d- glut + thiosulfate -

CG 7 & ~~CG 8~~^{CG 8} run-downs + sulfide. Heavy ppt causing turbidity in tubes. This medium was made in same salt mixture as that which gave little or no ppt in last batch of AG 2!

Started broths of CG 7, 8, & 9 and 2UVI for crossing attempts.

Also inoc 2UVI in both 7, 8, & 9.

7/23/54

CG 7 in run-downs; no growth (inoc 7/20)

Plated crosses of CG 7, 8, 9 & 2UVI

7/24/54

Inoc CG 7 into A1 run-downs; CG 8 into A1 & A2

7/26/54

CG 7 & 8 rundowns

	<u>CG 7</u>			<u>CG 8</u>		
	<u>2 days</u>	<u>3 days</u>	<u>4 days</u>	<u>2 days</u>	<u>3 days</u>	<u>4 days</u>
O	-	-	-	O	-	±
A1	+	++	++	A1	±	++
lys	-	-	+	lys	-	±
arg	-	±	±	arg	-	±
meth	-	-	-	meth	-	±
cyst	-	±	+	cyst	-	±
complete	+++	++++	++++	A2	+	+
				leuc	-	-
				isol	±	±
				val	-	-
				compl	+++	++++

This batch of minimal apparently not very satisfactory. Better growth in A2 at 2 days in previous test (AA rundowns)

Crossing attempts of CG 7, 8, & 9 \times 2UV1

- Controls: negative except CG 8, which is quite heavily contaminated w mold and a pink bacterial contaminant. A few col. present which look like Agrobacterium.
- CG 8 & 9 ^{+2UV1} cross plates: negative
- CG 7 \times 2UV1: grown together: 2 plates; negative
- grown separately: 1 plate; 61 colonies. Look like

Agrobacterium. Plate has some mold near edge, but bacterial col. evenly distributed on plate, do not seem to be stimulated by mold.

Pick colonies (at least 20); spot on complete, replicate to minimal. Repeat cross, this time grown together & separately, aerated & un-aerated.

7/27/54

CG 7 & 8 AA run-downs: (See table on previous page)

CG 8 seems rather "leaky"; this has happened before. CG 7: Might try A / single omissions. Also, try individual vitamins and the vitamin mix used in yeast medium, since the sulfide does not counteract very well the inhibition by the vitamin mix.

Next run-down attempt - 2UV1

Repeat l- & d- glutamic ppt \bar{c} CG 15, since previous ppt was lost because of mold.

Start cultures for repeat crossing attempt \bar{c} CG 7 & 2UV1.
(together & sep; aerated & un-aerated.)

7/28/54

Col. picked from 7x2 UV1 plate & repl. from complete 2
minimal: 29 picked, all prototrophs.

CG15 on d-glutamic: no growth in 24 hrs

7/29/54

Plated 7x2 UV1 crosses (grown together & separately,
aerated & not aerated)

[Some error in non-aerated cultures that labels
may have been interchanged on CG7 & CG7+2UV1

CG15 / glutamic:

	<u>2 days</u>	<u>3</u>	4
0	++	+++	
Na ₂ S only	++	+++	
d-glut	±	++	!
d-glut + Na ₂ S	++	+++	
l-glut	++	+++	
l-glut + Na ₂ S	++	+++	

Checking reveals the "d-glutamic" is actually l(+)
glutamic (Kodak). The other glutamic is l(+)-glutamic
(GBI).

7/30/54

CG 7 run downs

	<u>2 days</u>	<u>3 days</u>		<u>2 days</u>	<u>3 days</u>
O (Na ₂ SO ₄)	+	+	A1	++	+++
B ₂	+	+	"-lys	++	+++
Biotin	±	traced	"-arg	++	+++
B ₆	-	±	"-cys	-	++
pantoth	-	-	"-meth	+	+++
K	-	±			
B ₁	-	±	complete	+++	+++
nic	++	++ traced			
B ₁₂	-	±			
inos	-	±			
chol	++	contaminant? +++ (pullula)			
paba	-	±			
pge	-	-			

Streak out CG 7; check several colonies on anything giving 2+ or better.

7/31/54

CG 7 x 2UV1 "cross": At 48 hrs, colonies on all three plates from cultures grow together, not aerated. None on other plates.

Inoc. run downs (primary) of 2UV1

8/2/54

2 UV1 primary run-downs;

at 2 days, growth only in YNA.

Try individual vito; also p & p?

CG7: from individual colonies. Check each on
 min ($\frac{1}{2}$ sulfide) +: O; biotin; methionine; choline;
 A1 & individuals of A1 group. Try first 2 plates; 2 or three with

CG7 + 2 UV "cross": (4 days)

Colonies on all 3 plates grown together, not aerated.

(Counted 1 plate: 115 col; other 2 similar). Thin
 film of background, so could be feeding. Pick a few
 col, streak, test for prototrophy. Col. are small.

No real control on this tube; could be mutation to
 prototrophy.

Repeat cross again.

8/3/54

2 UV1 primary run-downs; 3 days; still growth
 only in YNA; possibly trace (\pm) on A1.

8/4/54

2 UV1 primary run-downs; 4 days

O	-	A3	-	HC	-
A1	\pm	A4	+	VITS	+
A2	-	A5	+	VFX	...

Try individual
 vito.

7x20V1 "protos" (8/2): Very poor growth when streaked on minimal - very tiny colonies. Grew well on complete. Replicate from complete to mini.

Repeat 7x20V1 cross: Use several cultures of each parent, plate control from each.

8/5/54

7x20V1 "prototrophs" - poor growth when replicated to minimal (24 hrs)

C67 from single col. repl. to mini. agar + supplements - 48 hrs. No growth except on complete

(Suppl. used: Protein, nic, choline, A1, & individuals of A1 group)

8/6/54

20V1 in individual vials: no growth at 2 days

Plated repeat of 7x20V1 cross.

8/9/54

Repeat of 7x2 UVI cross

Shew up several tubes of 7 and of 2 UVI, plated controls & cross from each (except 7-5 & 7-6, controls only). Also grew 2 tubes of 7+2 UVI together.

	<u>CG 7 control</u>	<u>2 UVI control</u>	<u>cross plate</u>
1	ca 200 col	1 col.	ca 200
2	7 col ^{may be contaminants}	0	several very small
3	0	confluent	confluent
4	1 ^{may be contaminants}	0	3 + several very small
5	0	—	—
6	10 very small	—	—
7+2 UVI ①	—	—	confluent
7+2 UVI ②	—	—	0

8/10/54

Check whether filtered Ag 2 supports good growth of CG 15 (in order to get rid of confusing turbidity).
Inc. tube (E Na₂S) & water suspension.

8/12/54

3 days growth in filtered medium only

9/13/54 3 days growth in both filtered & unfiltered,

Incubation very small results in no growth in control Ag 1 control tube until 48 hrs

9/7/54

Made up 40 tubes Ag 2 (2x) + 40 tubes
1% sucrose Ag 2 has no Fe, Mn, or Zn,
also no sulfide. Filtered before tubing &
autoclaving. Check all aseptics

1) in min

2) in min + sulfide

3) ~~in min + sulfide~~ + VE. Complete

before starting any apts.

9/13/54

Check of aseptics since into min + sulfide, &
complete 9/11:

No growth in any min or sulfide tubes.

Growth in all complete tubes except 2 UV2.

Continue inc.

9/14/54

Still no growth in any minimal; no growth
in 2 UV2 complete. Check again on viability
of 2 UV2 stab.

Transfer stabs to have one set as reserve.

9/15/54

Growth in CG7 in min + sulfide. Could be
contaminated, since another tube has mold.